DETAILED ACTION

1. This action is in response to the papers filed June 21, 2010. Currently, claims 1-30 are pending. Claims 7-12, 22-30 have been withdrawn as drawn to non-elected subject matter.

Election/Restrictions

2. Applicant's election without traverse of the species of T4141G Mutation and Alzheimer 's disease, Claims 6 and 21 in the paper filed June 21, 2010 is acknowledged.

In a telephone interview on August 17, 2010, the Examiner called Robert Buyan to request clarification regarding the T414**1**G mutation. The specification and the art teach a T414G mutation. In the telephone interview Mr. Buyan requested the examiner treat the election as T414G rather than T4141G. Moreover, Mr. Buyan indicated he would file an amendment to this effect in response to the office action. Therefore, in an effort to facilitate compact prosecution, an action on T414G has been prepared.

Claims 7-12, and 22-30 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim.

The requirement is still deemed proper and is therefore made FINAL.

Priority

3. This application claims priority as a 371 to PCT/US05/10266, filed March 29, 2005 and provisional application 60/557,612, filed March 29, 2004.

Drawings

4. The drawings are acceptable.

Sequence Rules

5. This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825.

The specification contains sequences which are not identified by SEQ ID NO:. For example, page 10 contains four primers that fail to have an identifier. Appropriate correction is required.

Specification

6. The specification refers to a reference by Murdock on page 3 of the specification. The citation appears to be incorrect. The Murdock et al, Nucleic Acids Research, Vol. 28, pages 4350-4355 paper appears to have been published in 2000 rather than 2002. Appropriate correction is requested.

Claim Objections

7. Claims 2-5 are objected to because of the following informalities: the claims recite mtDNS. It appears that mtDNS contains a typo and should read mtDN**A**. Claims 4 and 5 contain multiple recitations of mtDNS. Appropriate correction is required.

Claim Rejections - 35 USC § 112-Description

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. Claims 1-3, 13-21 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are broadly drawn to methods requiring determining the presence of mtDNA CR mutations.

The specification teaches the mtDNA CR is a 1000 nucleotide pair, non-coding, region of the mtDNA (page 2, lines 23-25). The specification teaches 6 mtDNA CR mutations.

Ariad Pharmaceuticals Inc. v. Eli Lilly & Co., 94 USPQ2d 1161 (Fed. Cir. 2010) recently re-affirmed the written description requirement. *Ariad* reiterates that "the hallmark of written description is disclosure" and "possession as shown in the

disclosure" is a more complete formulation of the test for written description. *Ariad* considers situations of genus claims and states that the written description requirement ensure that "when a patent claims a genus by its function or result, the specification recites sufficient materials to accomplish that function."

Vas-Cath Inc. V. Mahurkar, 19 USPQ2b 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed". Applicant is reminded that *Vas-Cath* makes clear that the written description provision of 35 USC 112 is severable from its enablement provision. In *The Regents of the University of California v. Eli Lilly* (43 USPQ2b 1398-1412), the court held that a generic statement which defines a genus of nucleic acids by only their functional activity does not provide an adequate written description of the genus. The court indicated that while Applicants are not required to disclose every species encompassed by a genus, the description of a genus is achieved by the recitation of a representative number of DNA molecules, usually defined by a nucleotide sequence, falling within the scope of the claimed genus. At section B(1), the court states that "An adequate written description of a DNA…' required a precise definition, such as by structure, formula, chemical name, or physical properties', not a mere wish or plan for obtaining the claimed chemical invention".

With respect to claims which encompass variants, as provided in Example 7 of the Written Description Guidelines, no common structural attributes identify the members of the genus. The current claims encompass a large genus of nucleic acid

mutations which comprise variants in any region of the mtDNA control region (CR) nucleic acid. The genus includes an enormous number of variants, polymorphisms and mutations for which no written description is provided in the specification. This large genus is represented in the specification by only the particularly named 6 polymorphisms. The art teaches that "variants" refers to a gene or gene product that displays modifications in sequence and/or functional properties (altered characteristics) when compared to the wild-type gene. This genus encompasses SNPs, deletions, insertions, for example.

The general knowledge and level of skill in the art do not supplement the omitted description because specific, not general guidance is what is needed. Since the disclosure fails to describe the common attributes or characteristics that identify members of the genus, and because the genus is highly variant, variants of the control region of mtDNA alone is insufficient to describe the genus. There is no description of the mutational sites that exist in nature and there is no description of how the structure of the control region mtDNA relates to the structure of any strictly neutral alleles. The general knowledge in the art concerning variants does not provide any indication of how the structure of one allele is representative of unknown alleles. The nature of alleles is such that they are variant structures, and in the present state of the art the structure of one does not provide guidance to the structure of others. The common attributes are not described. The specification provides no correlation between structure of polymorphisms and the function of such polymorphisms. The polymorphisms shown are not representative of the genus of any polymorphism associated with development

of beta amyloid deposits or fibrils or assessing the efficacy of treatment rendered to a subject for such disorder because it is not clear which polymorphisms within the mtDNA would have the same effect. One of skill in the art would conclude that applicant was not in possession of the claimed genus because a description of only six members of this genus is not representative of the variants of the genus and is insufficient to support the claim. Accordingly, Applicants have not adequately disclosed the relevant identifying characteristics of a representative number of species within the claimed genus.

Claim Rejections - 35 USC § 112-Scope of Enablement

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. Claims 1-6, 13-21 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for determining the presence of mtDNA CR mutations, does not reasonably provide enablement for a method for diagnosis of any disorder associated with the development of beta amyloid deposits or fibrils, such as Alzheimer's disease, in a human or animal by determining the presence or quantity of mtDNA CR mutations, such as T414G. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC 112, first paragraph, have been described by the court in *In re Wands*, 8 USPQ2d 1400 (CA FC 1988). *Wands* states at page 1404,

> "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The nature of the invention and breadth of claims

The claims are drawn to methods for diagnosis of any disorder associated with the development of beta amyloid deposits or fibrils, such as Alzheimer's disease, in a human or animal by determining the presence or quantity of mtDNA CR mutations, such as T414G.

The claims are also directed to quantifying the number of mtDNA CR mutations and comparing a mtDNA CR value to control or diseased values to diagnose a disease.

Claims to diagnosis require a reliable association between the genotype and the phenotype.

The invention is in a class of invention which the CAFC has characterized as "the unpredictable arts such as chemistry and biology." Mycogen Plant Sci., Inc. v. Monsanto Co., 243 F.3d 1316, 1330 (Fed. Cir. 2001).

The unpredictability of the art and the state of the prior art

The art teaches the detection of mtDNA control region mutations as a diagnostic for disorders associated with the development of beta amyloid deposits or fibrils in a human or animal is unpredictable at the time the invention was made.

Murdock et al. (Nucleic Acids Research, Vol. 28, No. 21, pages 4350-4355, 2000) teaches age-related accumulation of the T414G mitochondrial DNA control region mutation in muscle, but not in brain. Murdock analyzes the mtDNA using a sensitive PNA-directed PCR clamping based method (limitations of Claim 1-3). In particular the T414G mtDNA mutation was analyzed in both human skeletal muscle and brain samples for the accumulation of the mutation with age (page 4351, col. 1). The relative quantities of mtDNA were measured using competitive PCR (page 4351, col. 2). As seen in Figure 2, PNA-clamping blocks wild-type, but not mutant, molecule amplification to prevent false positive amplification (page 4352, col. 1). To increase the sensitivity of the PNA-clamped reaction, a second round of PCR on diluted product from the first reaction may be performed using restriction enzyme digestion (page 4352, col. 2-4353, col. 1). Murdock concludes that PNA-clamped reactions can be multiplexed to allow simple and efficient identification of multiple mtDNA mutation in diagnosis of mtDNA disease (page 4351, col. 2). The PNA-clamping also permits low levels of heteroplasmy mutations to be detected at a ratio of 1:100. Thus, the control region mtDNA mutation T414G was found in skin fibroblasts from older human subjects and also accumulates in skeletal muscle after age 35 years (page 4354, col. 1). The T414G mutation could not be detected in any human brain sample, even from subjects as old as 93 years (page 4354, col. 1). Murdock teaches each active method step required by the instant claims.

Chinnery et al. (Am. J. Hum. Genetics, Vol. 68, pages 529-532, published electronically December 21, 2000) teaches determining the presence of mtDNA control region (CR) mutations. In particular Chinnery studied the mtDNA control region in brain

tissue from 31 normal elderly individuals, from 35 individuals who had Alzheimer disease and from 47 individuals who had dementia with Lewy bodies. Chinnery teaches postmortem control brain tissue was collected. Chinnery teaches sequencing nucleotides 33-785 of the control region which included the T414G mutation using primer extension reactions and electrophoresis (page 530, col. 1-2). Chinnery fails to detect the T414G polymorphism in any of the 113 samples of brain DNA, either control or individuals with AD. It is unpredictable how the skilled artisan would diagnose AD based upon the T414G polymorphism.

Simon et al. (Genomics, Vol. 73, pages 113-116, 2001) teaches screening for the T414G mutation in brain-derived mtDNA from 8 Alzheimer's disease patients, 27 Parkinson's disease patients, 4 multiple system atrophy patients and 44 controls. Simon failed to detect the T414G mutation in any of the cases (abstract). Simon teaches sampling brain samples from 4 different regions of the brain (page 114, col. 2). Simon also analyzed blood and fibroblasts and the T414G mutation was absent from these tissues also. Simon suggests that there is a possibility that the T414G mutation may be present in brain regions not examined in their study (page 115, col. 1).

Coskun et al. (PNAS, Vol. 101, No. 29, pages 10726-10731, July 20, 2004), applicant's own work, finds that 65% of the AD brains harbored the T414G mutation whereas this mutation was absent from all controls (abstract). Coskun acknowledges the T414G mutation was not detected by others using less sensitive primer extension strategies such as Chinnery.

Howell (Trends in Genetics, Vol. 21, No. 11, pages 583-586, November 2005) considers previous studies performed by Chinnery and Coskun and concludes that the role of mtDNA mutations in the development of AD or PD still remains unestablished (abstract). When considering the results by Coskun, Howell states that the new results

of Coskun are inconsistent with previous findings. Howell suggests that the study by Chinnery involved larger number of tissue samples and did not detect the mutation in brains, AD patients or those with dementia and Lewy bodies. Howell suggests the inconsistent findings may be due to different samples of brain tissue. Howell also considers the findings of higher levels of mutations which were not observed by Chinnery. Howell further considers the scientific concerns that mtDNA point mutations are random and independent, how a heteroplasmic somatic mtDNA mutation can reach high levels in the brain tissue of these patients. Howell states that purely on statistical grounds the chances of an mtDNA mutation arising early in the cell lineage that gives rise to the brain will be extremely low. Howell states it is difficult to envisage such a chance event occurring often enough in the human population to account for the prevalence of AD (page 584, col. 2).

Guidance in the Specification.

The specification teaches amyloid fibrils are thought to be involved in the pathogenesis of various amyloid diseases of genetic, infectious and/or spontaneous origin including Alzheimer's disease, spongiform encephalopathies, Parkinson's disease, type II diabetes, Creutzfeldt-Jakob disease, Down's syndrome associated dementia, Huntington's disease, macular degeneration various prion diseases and numerous others. This is a very diverse collection of diseases.

The specification teaches that the mtDNA control region (CR) is a 1000 nucleotide pair, non-coding, region of the mtDNA that contains the promoters for the initiation of heavy (H) and L-strand transcription (PH & PL) (page 2 of the specification). The mtDNA CR encompasses the light (L) - and heavy (H) strand promoters (Pl and Ph), mtTFA, CSB I, II, and II, Oh1 and Oh2 (page 2 of the specification).

The specification analyzes the total number of heteroplasmic mtDNA CR mutations observed by cloning and sequencing CR clones from AD and control brain samples (page 5, lines 7-10). Figure 3A illustrates the differences between AD and control brains to demonstrate a significant difference. Figure 3B illustrates the difference in patients 80 years and up is significant.

With respect to analysis of the T414G mutation, Example 1 tests for the mutation by PNA clamping PCR in AD brain frontal cortex (page 6 of the specification). The specification sampled a total of 23 AD and 40 control (non-AD) brains (page 9 of the specification). The mutation was found in 65% of the AD brains while none of the normal control brains had the mutation.

The guidance provided by the specification amounts to an invitation for the skilled artisan to try and follow the disclosed instructions to make and use the claimed invention.

Quantity of Experimentation

The quantity of experimentation in this area is extremely large since there is significant number of parameters which would have to be studied to enable the skilled artisan to practice the claimed invention as broadly as claimed.

First the claims are directed to any disorder associated with the development of beta amyloid deposits or fibrils. The specification teaches the genus of disorders encompasses genetic, infectious and/or spontaneous origin including Alzheimer's disease, spongiform encephalopathies, Parkinson's disease, type II diabetes, Creutzfeldt-Jakob disease, Down's syndrome associated dementia, Huntington's disease, macular degeneration various prion diseases and numerous others (see page 2 of the specification). The art teaches the CR mutations are not associated with Lewy

bodies and dementia (see Chinnery, 2000). There is no evidence that diabetes type II patients have any CR mutations at a statistically significant level. The instant specification fails to provide any evidence that all of these disorders are predictably associated with CR mutations. It is unpredictable that each of these distinct disorders is similarly associated with CR mutations absent further unpredictable and undue experimentation.

Second, the claims are directed to both humans and animals. The specification and the art appear to be focused on humans. There are no teachings in the art or the specification whether animals may be diagnosed with disorders associated with the development of beta amyloid deposits or fibrils based upon mtDNA CR mutations. It is unpredictable which mutations, if any mutations are present in animals such as dogs, cat, chimps, rabbits, for example. It is further unpredictable whether these animals accumulated mtDNA mutations in the same manner as humans. Without further, unpredictable and undue experimentation the skilled artisan would be unable to make any diagnosis for dogs, cats, rabbits, chimps regarding mtDNA CR mutations.

Third, with regard to the detection of the mtDNA CR T414G mutation, the prior art teaches the T414G mutation is found in both young and older individuals' fibroblasts (see Michikawa). Murdock teaches the T414G mutation could be detected in muscle from individuals 30 years and older. This suggests that the T414G mutation is found in normal, non diseased individuals. It would be unpredictable that the mere detection of T414G would be indicative of a disorder associated with the development of beta amyloid deposits or fibrils in humans. If the claims were limited to brain tissue samples, it is unpredictable how the skilled artisan could obtain brain tissue to be able to diagnose an individual prior to post mortem in an effective manner.

Fourth, with regard to the particularly elected embodiment of detecting T414G mutation as indicative of Alzheimer's disease, the prior art teaches a lack of association of the mutation with AD. Murdock teaches that the T414G mutation could not be detected in any human brain sample, even from subjects as old as 93 years (page 4354, col. 1). Chinnery fails to detect the T414G polymorphism in any of the 113 samples of brain DNA, either control or individuals with AD. Simon also analyzed brain-derived mtDNA for the T414G mutation from 8 Alzheimer's disease patients, 27 Parkinson's disease patients, 4 multiple system atrophy patients and 44 controls but failed to detect the T414G mutation in any of the cases. It is unpredictable how the skilled artisan would diagnose AD based upon the T414G polymorphism since the art teaches the T414G mutation is not found in brain tissue or more specifically brain tissue from AD or Parkinson's disease patients. The post-filing date art reviews that studies from Chinnery and Coskun (applicant's work) and concludes the role of mtDNA mutations in the development of either AD or PD still remains to be established. Howell expresses scientific concerns with the frequency at which Coskun detected the mtDNA mutations. In particular Howell stated that the results were inconsistent with previous findings that involved larger number of tissue samples. Howell also rationalized that "purely on statistical grounds, the chances of an mtDNA mutation arising early in the cell lineage that gives rise to the brain will be extremely low. It is difficult to envisage such a chance event occurring often enough in the human population to account for the prevalence of AD." Thus, given the inconsistent results and the scientific rationale provided by Howell, it is unpredictable that the skilled artisan could diagnose AD using the T414G mutation absent further unpredictable and undue experimentation. This would require significant inventive effort, with each of the many intervening steps, upon

effective reduction to practice, not providing any guarantee of success in the succeeding steps.

Level of Skill in the Art

The level of skill in the art is deemed to be high.

Conclusion

In the instant case, as discussed above, in a highly unpredictable art where the art teaches the unpredictability of detecting mtDNA CR mutations for diagnosis disorders. Further, the prior art and the specification provides insufficient guidance to overcome the art recognized difficulties of diagnosing disorders based upon mtDNA CR mutations. Thus given the broad claims in an art whose nature is identified as unpredictable, the unpredictability of that art, the large quantity of research required to define these unpredictable variables, the lack of guidance provided in the specification, the absence of a working example and the negative teachings in the prior art balanced only against the high skill level in the art, it is the position of the examiner that it would require undue experimentation for one of skill in the art to perform the method of the claim as broadly written.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

10. Claims 1-4, 6, 14-17 are rejected under 35 U.S.C. 102(b) as being anticipated by Michikawa et al. (Science, Vol. 286, pages 774-779, October 22, 1999).

The only active method step in Claim 1 requires determining the presence of mtDNA CR mutations.

Michikawa teaches analyzing mtDNA and finding a high copy point mutations at specific positions in the control region (abstract). The sensitive method for detection of aging-related heteroplasmic mutation in the mtDNA control region relied on enzyme digestion and DGGE in combination with cloning (page 775, col. 1)(limitations of Claims 16). Michikawa teaches the T414G transversion was found in up to 50% of mtDNA molecules of individuals above 65 years of age but was absent in 13 younger individuals. Michikawa teaches that the T414G was not precisely quantifiable, but could be detected when particularly abundant by direct DNA sequencing of the PCR product and by allele specific termination by primer extension (page 777, col. 2)(limitations of Claims 2, 3, 6). Primer extension requires oligonucleotide hybridization followed by extension (limitations of Claims 14, 15). Michikawa teaches the number of mtDNA mutations was compared to those of “young” individuals. The T414G transversion was not found in any control individuals (page 777, col. 1). Thus, Michikawa compared the quantity with a control and "older" individuals had more mtDNA CR mutations than the control (limitations of Claim 4). Michikawa teaches analysis of mtDNA from fibroblast cultures (limitations of Claim 17). Michikawa teaches each active method step required by the instant claims.

11. Claims 1-3, 6, 13-17 are rejected under 35 U.S.C. 102(b) as being anticipated by Murdock et al. (Nucleic Acids Research, Vol. 28, No. 21, pages 4350-4355, 2000).

The only active method step in Claim 1 requires determining the presence of mtDNA CR mutations.

Murdock teaches age-related accumulation of the T414G mitochondrial DNA control region mutation in muscle, but not in brain. Murdock analyzes the mtDNA using a sensitive PNA-directed PCR clamping based method (limitations of Claim 1-3). In particular the T414G mtDNA mutation was analyzed in both human skeletal muscle and brain samples for the accumulation of the mutation with age (page 4351, col. 1)(limitations of Claim 6, 17). The relative quantities of mtDNA were measured using competitive PCR (page 4351, col. 2). As seen in Figure 2, PNA-clamping blocks wild-type, but not mutant, molecule amplification to prevent false positive amplification (page 4352, col. 1)(limitations of Claim 13-15). To increase the sensitivity of the PNA-clamped reaction, a second round of PCR on diluted product from the first reaction may be performed using restriction enzyme digestion (page 4352, col. 2-4353, col. 1)(limitations of Claim 16) Murdock concludes that PNA-clamped reactions can be multiplexed to allow simple and efficient identification of multiple mtDNA mutation in diagnosis of mtDNA disease (page 4351, col. 2). The PNA-clamping also permits low levels of heteroplasmy mutations to be detected at a ratio of 1:100. Thus, the control region mtDNA mutation T414G was found in skin fibroblasts from older human subjects and also accumulates in skeletal muscle after age 35 years (page 4354, col. 1). The T414G mutation could not be detected in any human brain sample, even from subjects as old

as 93 years (page 4354, col. 1). Murdock teaches each active method step required by the instant claims.

12. Claims 1-3, 6, 14-15, 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Chinnery et al. (Am. J. Hum. Genetics, Vol. 68, pages 529-532, published electronically December 21, 2000).

The only active method step in Claim 1 requires determining the presence of mtDNA CR mutations.

Chinnery teaches determining the presence of mtDNA control region (CR) mutations. In particular Chinnery studied the mtDNA control region in brain tissue from 31 normal elderly individuals, from 35 individuals who had Alzheimer disease and from 47 individuals who had dementia with Lewy bodies. Chinnery teaches postmortem control brain tissue was collected (limitations of Claim 17). Chinnery teaches sequencing nucleotides 33-785 of the control region which included the T414G mutation using primer extension reactions and electrophoresis (page 530, col 1-2)(limitations of Claim 6, 14-15). Chinnery teaches each active method step required by the instant claims.

Conclusion

13. No claims allowable.

14. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

a. Wang et al. PNAS, Vol. 98, No. 7, pages 4022-4027, March 27, 2001.

Wang teaches the T414G mutation was not present in muscle.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (571) 272-0743. The examiner can normally be reached Monday-Friday from 7:00 a.m. to 4:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, David Nguyen, can be reached on (571)272-0731.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

The Central Fax Number for official correspondence is (571) 273-8300.

/Jeanine Goldberg/
Primary Examiner
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